NATIONAL INSTITUTES OF HEALTH

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

CYSTIC FIBROSIS

Cystic fibrosis (CF) is the most common lethal genetic disease in Caucasians. Approximately 1 in 2,000 newborns have the disease, which causes a combination of digestive and lung problems. Without intensive treatment, CF can be fatal within a few years. However, with improved diagnosis and more sophisticated treatment available today, many children with CF now live into their twenties and beyond.

CF clearly remains a serious problem and a difficult scientific challenge. While treatment has improved, the disease remains incurable. Research over the years has provided a very thorough description of the features of the disease and the major and minute tissue abnormalities that might serve as clues to the fundamental inherited CF defect. Only recently, however, have scientists found an abnormality that can be demonstrated in a variety of tissues affected by CF. The greatest hope now lies in the use of genetic and molecular biological techniques to pinpoint the biochemical defect that underlies the symptoms of CF.

The Disease

The term cystic fibrosis refers to changes in the pancreas that were noticed by physicians before the disorder was fully described. These early observers noticed cysts and fibrous scarring in the pancreas, a gland just below the stomach. The reason for this scarring is that in CF, the pancreas' secretions into the digestive tract are thicker than usual and can damage the gland by blocking drainage. The pancreas is an exocrine gland--its digestive enzymes are secreted into ducts through which they pass into the digestive tract. (The pancreas also produces endocrine hormones, like insulin, which are secreted into the bloodstream.) CF characteristically affects the secretions of all the body's exocrine glands--the sweat and salivary glands and those that secrete mucus such as those lining the airways in the lungs and trachea. Mucous secretions are thickened and copious. The thickening of pancreatic secretions results in digestive difficulties in children with CF. Even with an ample diet, these children can be malnourished.

While the lungs of a newborn with CF are normal, over the years the accumulation of mucus makes it difficult for the child to clear his or her lungs of mucus and foreign matter. Coughing becomes chronic, and children with CF are particularly susceptible to bacterial infections of the lung.

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The sweat of children with CF is saltier than normal. This feature forms the basis of the current diagnostic test for CF. The sweat test measures salt in sweat and provides a very accurate diagnostic tool.

Treatment for CF at this time is aimed at the secondary effects of the disease. Pancreatic insufficiency is treated with enzyme supplements. Lung disease is treated with a combination of chest physical therapy to loosen lung mucus and antibiotics to control bacterial infections.

There are an estimated 1,000 to 2,000 new cases of CF each year in this country and perhaps 33,000 people with the disease. Five percent of the population, or about 12 million Americans, are symptomless carriers of the disease. If two carriers marry, each of their children has a one in four chance of having CF and a one in two chance of being a carrier.

CF is a costly disease to treat. Hospitalization contributes to the yearly cost of treatment, estimated at about $10,000. Young adults with CF are still vulnerable to the life-threatening lung disease associated with CF. This fact can make planning for the future--from a personal and a professional point of view--difficult. In addition, fertility can be compromised in young men with CF, and pregnancy can threaten the health of young women with the disease.

Research

Research over the years on CF has had two aims: first, improving treatment of the lung disease and digestive effects; and second, identification of the underlying cause. As mentioned previously, early diagnosis and the use of physical therapy and antibiotics have allowed many children to live into their twenties. There may, however, be a limit to how much more increase in lifespan and quality of life is possible through treatment of the secondary effects of this disease. The key to curing CF lies in the identification of the disease's underlying cause for which, in the past, there have been very few helpful clues.

Within recent years, the development of new research techniques and the resulting availability of additional tools with which to explore CF have brought a refocusing of scientific attention on this puzzling disease. In addition, scientists with specialty training in diverse scientific areas are bringing their expertise to CF. The clear pattern of inheritance in CF makes this a disease for which new genetic techniques can be used to pinpoint the familially transmitted defect.

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Tissue Abnormalities in CF

Much current research on CF is based on an earlier advance that was a product of research on the excessive saltiness in the sweat of CF patients. Dr. Paul Quinton, a grantee of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK), at the University of California, Riverside, reported a defect in permeability to the chloride ion in isolated sweat ducts. Chloride, part of the sodium chloride compound that is table salt, is important in fluid balance in the body. The defect in permeability to chloride has since been confirmed in cells that line the airways, the nasal passages, and the trachea. Scientists hope that identification of the cause of this defective permeability may provide the avenue for determining at a cellular level how the disease does its damage.

In continuing work, Dr. Quinton has tested whether sweat from CF patients can affect the secretory properties of normal sweat glands. Many scientists have postulated and sought the existence of a CF "factor" in the exocrine secretions of CF patients that would cause the alterations in tissue function characteristic of the disease. Dr. Quinton reported no such measurable effect of sweat on normal gland function, narrowing the range of options by which a biochemical defect might act to cause CF.

Scientists are also looking at the neurologic control of sweat glands. Research conducted by Dr. Kenzo Sato at the University of Iowa, Iowa City, has demonstrated that in CF, sweat glands do not respond normally to nervous system stimulation that is usually important in controlling the function of the glands. Responsiveness of other organ systems to this kind of stimulation, or sympathetic nervous system control, has also been observed to be abnormal in people with CF, another clue that there is a generalized defect in sympathetic nervous system responsiveness in CF.

If so, the next step would be to determine how such a defect would originate. The sympathetic nervous system characteristically acts through a "second messenger" or intermediary molecule, cyclic AMP. Dr. Sato's research has shown that isolated sweat glands from CF patients accumulate a normal amount of cyclic AMP in response to sympathetic nervous system stimulation. This finding suggests that the abnormality in the neurologic response of the sweat gland may not result from a defect in accumulation of cyclic AMP, but in a later step in the chain of biochemical events leading to sweat secretion.

Calcium is another intracellular messenger that can influence a cell's metabolic responses. Scientists are conducting research to characterize the role of calcium in metabolic control and to determine whether calcium turnover is abnormal in CF. For example, Dr. Dorr Dearborn and colleagues

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at the Cystic Fibrosis Center at Case Western Reserve University, Cleveland, have looked at calcium distribution in cells from CF patients. They found that total intracellular calcium is about 25 percent higher in CF cells than in cells from individuals without CF. Half of this calcium is contained in mitochondria, cell organelles that have an important function in energy metabolism. Continuing research will be aimed at identifying what the defect in calcium metabolism is and how it may contribute to an abnormality in metabolic control in these tissues.

The defects described above reside in the cells that line the surfaces of exocrine glands and ducts. In order to study the properties and defects of these so-called epithelial cells, cell culture techniques have to be expanded to provide a constant supply of healthy cells for experimentation. NIADDK sponsored a workshop in July 1985, bringing together experts in research on CF epithelial tissues and tissue cell culturing to promote collaboration in the development of these techniques. Among the scientists who discussed the tissue culture techniques developed for tissues affected by CF were NIADDK grantees Dr. Donald Sens, of the Medical University of South Carolina, Charleston, who is studying sweat gland duct cells and Dr. Carol Liedtke, Case Western Reserve CF Center, who is studying rabbit tracheal epithelial cells in culture.

The groundwork for current cell culture techniques has been laid by years of basic research. The technologies for isolating, culturing, and manipulating cells have advanced tremendously and have made possible the studies of ion transport and neurologic response described above.

Genetics

Another approach to identifying the CF defect, and one that is now receiving great emphasis, is to search for the defective gene. The protein product of the gene can then be determined using the genetic code, and the effects of this protein on cell function can be studied. This research has gained momentum within the last year. NIADDK has actively solicited research applications for grants to study the genetics of CF, and has funded several new studies in the past year. The principal investigators on these projects bring their expertise in cell genetics to a field that needs and lends itself to this kind of investigation.

These opportunities in genetics are only now becoming available because of recent advances in techniques that allow scientists to screen for genetic anomalies and determine whether they are linked to CF. CF provides a good opportunity for this kind of investigation because the pattern of its inheritance is clear. It is inherited as a single recessive trait, that is, a child must inherit the gene from both parents to have the disease.

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An NIADDK-supported investigator, Dr. Lap-Chee Tsui, at the Hospital for Sick Children in Toronto, has just discovered a marker that appears to be inherited along with the CF gene with a high degree of frequency. At the same time, another NIADDK-supported investigator, Dr. Katherine Klinger, at Integrated Genetics, Rochester, New York, has found evidence that another marker was transmitted with the CF gene. These preliminary reports suggest that research in this area has the potential for exciting new discoveries.

In addition to supporting research of this type, NIADDK has initiated a collaborative program with the Department of Energy (DOE) under which its laboratories at Los Alamos, New Mexico, will be making specially prepared filter paper sets impregnated with pieces of DNA from each human chromosome. This central resource will spare independent investigators from having to acquire the equipment and personnel to prepare such material themselves, saving duplication of effort and funds. The filter paper can be used repeatedly to determine on which chromosome a given gene marker is located. This resource should be useful for research on CF and a host of other genetic disorders.

NIADDK is also funding another resource that will facilitate genetic research on CF. The Human Genetic Mutant Cell Repository in Camden, New Jersey, is collecting blood cells from families with CF to provide DNA for genetic studies. This program is described more fully later in this report by the National Institute of General Medical Sciences (NIGMS).

Genetic markers for diseases can be used for prenatal screening and postnatal diagnosis. Ultimately, however, the search for genetic markers is an avenue to finding the CF gene and developing a treatment. For example, the CF mutation may result in the absence of an essential protein or the presence of a harmful one. Treatment in the first case might be to replace the missing protein, and in the second case to suppress the abnormal gene with drug therapy. The development of therapy directed at the gene depends on continued technological advances. In the future, such therapy might seek to clone and introduce normal genes into CF-affected tissues.

NIADDK Intramural CF Program

A recent reorienting of NIADDK's intramural research on CF has led to an interdisciplinary program of basic research on many aspects of the disease: ion transport; mucus and exocrine secretory problems; infections and immunology; genetics; and the structure and metabolism of glycoproteins, compounds that are important in the structure of mucus and immune substances. Under NIADDK's new CF Fellowship Program, physicians with an interest in CF are recruited for a 1- to 2-year fellowship in a specific area of basic research. The Cystic Fibrosis

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Foundation is aiding this program with grants that supplement the salary of each research fellow, making it possible to attract well-qualified individuals. The scientists collaborate with NIADDK scientists on basic research related to CF. This program started in the spring of 1984, and the research is well under way.

Dr. Richard Ornberg is an NIADDK intramural scientist who is doing research related to CF. He is using an analytical electron microscope recently developed by National Institutes of Health scientists in the Division of Computer Research and Technology and the Division of Research Resources to gather information on how cells secrete substances. With the analytical microscope, one of the most sophisticated in the world, Dr. Ornberg can visualize changes in distribution of chemical elements within a secretory cell's storage granule as it resides in the cell under living conditions. This information promises to help contribute to an understanding of exocrine secretory processes and the defects in secretion that occur in CF.

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National Heart, Lung, and Blood Institute

As discussed in the previous section, the fundamental flaw in CF may involve the movement of certain ions--notably chloride--across cell membranes in various tissues. Chloride ions move into or out of cells through designated channels in the cell membrane, always in the direction of favorable electrochemical gradients much as water in a stream follows the streambed and always flows downhill. According to some investigators, in CF patients there appears to be some malfunction or structural defect in the chloride channel that impedes the movement of this ion.

Dr. Richard Boucher and coworkers, National Heart, Lung, and Blood Institute (NHLBI) grantees at the University of North Carolina in Chapel Hill, studied ion movement in epithelial cells obtained from nasal polyps that had been removed from CF patients or from hay-fever sufferers. Under the conditions of the experiment, chloride ion movement across the membranes of cells from the CF patients was only half that seen in cells from the control individuals. Other evidence strongly indicated that the impedence of chloride movement in the CF cells was not due to any local or circulating mediator substance, but rather was intrinsic to the cells themselves. Similar impedence of chloride movement has been found by other investigators in tracheal cells and in sweat ducts from CF victims. In all these instances the cells exhibiting the chloride transport abnormality were from tissues known to be adversely affected by CF.

Of the disease-causing agents that take up residence in the lungs of CF patients--and which are often difficult and sometimes impossible to dislodge even with vigorous therapy--the bacterium Pseudomonas aeruginosa can be especially troublesome. Dr. Kenneth B. Alder and coworkers, NHLBI-supported investigators at the University of Vermont in Burlington, report that apart from the toxin this organism produces, it also elaborates an extracellular substance that, when added to a medium containing guinea pig or human tracheal tissues, stimulates them to produce and release increased quantities of mucin, the major component of mucus. This occurs in the absence of any detectable structural or toxic changes in the secretory cells of these tissues or any proliferation of their mucosa. The investigators think that it may also happen in the lungs of CF patients with P. aeruginosa infections and may well be an important factor in the excessive mucus that clogs their airways and fosters bacterial growth and replication. The investigators wonder whether this otherwise uncommon respiratory organism might not be helping to create an environment that enhances its own survival in CF patients.

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With more CF patients surviving through their twenties and into their thirties, cor pulmonale--heart disease resulting from lung disease--has become an increasingly prevalent complication of CF. Oxygen deficiency resulting from CF-associated lung problems may cause blood pressure in the pulmonary circulation to increase. In turn, this increases the circulatory burden of the heart's right ventricle, which may cause it to enlarge and, ultimately, to fail. Presently, the treatment of choice for cor pulmonale in CF is oxygen therapy, which selectively dilates pulmonary blood vessels, reduces pulmonary pressure, and improves oxygen delivery to organs and tissues; but the search continues for better therapeutic regimens for these patients.

Blood vessel dilators have been used with some success to treat pulmonary hypertension and cor pulmonale in patients with chronic obstructive lung disease. Therefore, Dr. Robert L. Geggel and coworkers, NHLBI grantees at Children's Hospital Medical Center in Boston and Harvard Medical School in Cambridge, Massachusetts, tested several of these dilators (phentolamine, nifedipine, and hydralazine) in patients with cor pulmonale due to CF, comparing the results obtained with the drugs to those obtained with oxygen therapy. None of the drugs was nearly as effective as oxygen therapy. Though hydralazine did improve heart performance and oxygen delivery somewhat, none had any significant effect on the pulmonary hypertension in these patients. The investigators conclude that oxygen therapy remains the treatment of choice for cor pulmonale secondary to CF, and that blood vessel dilators cannot be recommended unless subsequent study shows that the modest effects of hydralazine on heart performance and oxygen delivery favorably affect the prognosis in such patients.

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National Institute of Dental Research

Among the effects of CF are abnormal changes in salivary glands. Saliva plays a key role in protecting the mouth against infection. It also lubricates the oral tissues, helping to make food easier to swallow.

Saliva is normally a clear, free-flowing liquid. The saliva of CF patients, however, may be thick, sticky, and characterized by extremely viscous secretions. This abnormal saliva can block the salivary ducts, causing the glands to enlarge and become vulnerable to infection. It may also contribute to functional problems such as difficulty of speech and swallowing and may increase the risks of dental and oral soft-tissue disease.

The National Institute of Dental Research (NIDR) conducts and supports extensive study of the mechanisms that normally control exocrine gland secretion in order to determine what goes awry in disorders such as CF. NIDR intramural investigator Dr. Bruce Baum has used animal salivary glands to study the mechanisms controlling saliva formation. By focusing ohow the involuntary nervous system regulates secretions under normal conditions, abnormal conditions, and during aging, Dr. Baum and his coworkers hope to gain a better understanding of how salivary glands fail in patients with CF.

Saliva contains a broad spectrum of proteins that are known to play a pivotal role in maintaining the health of hard and soft oral tissues. The parotid gland--a major salivary gland--secretes many antibacterial proteins which carry sugar residues and are called N-linked glycoproteins. Dr. Eleni Kousvelari heads a team of investigators at NIDR who are studying the mechanisms involved in the processing, synthesis, and secretion of N-linked glycoproteins. They have discovered that alterations in chemical signals from nerves can affect how these glycoproteins are made and how they function.

Knowledge of the intracellular events associated with the secretory process may provide valuable insight into diseases such as CF. Dr. Eileen Watson of the University of Washington School of Dentistry in Seattle is conducting an NIDR-supported study of the mechanisms by which calcium ions regulate the secretory process. She is investigating the calcium-binding protein calmodulin, sodium ions, and cyclic AMP, as well as the relationship between calcium and these chemical messengers.

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In another extramural project, Dr. James W. Putney, Jr., of the Medical College of Virginia in Richmond is studying the pharmacology of salivary secretion. In laboratory studies, Dr. Putney is defining the pattern and sequence of signals transmitted from the cell surface and within the cell that are necessary to trigger the production and secretion of saliva. Studies of this kind, which define the cellular and molecular mechanisms of salivary secretion, improve understanding of the role of salivary secretion in dental health and define basic exocrine functions as they relate to CF.

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National Institute of Allergy and Infectious Diseases

Patients with CF are particularly susceptible to life-threatening respiratory infections. Through its extensive research programs, the National Institute of Allergy and Infectious Diseases (NIAID) supports studies aimed at preventing and controlling some of the major respiratory problems that affect CF patients.

Pulmonary symptoms of CF include excessive mucus secretion, recurrent infection and inflammation, and irritable air passages. It is, therefore, of vital importance to define factors that may result in these symptoms. NIAID grantee Dr. Robert E. Lemanske, Jr., at the University of Wisconsin-Madison has observed that similar, albeit less severe symptoms, occur in patients with allergic asthma. Like these asthmatics, CF patients have an increased incidence of positive skin tests for the presence of allergies. These important findings suggest an association between allergy and the pulmonary symptoms seen in patients with CF and those with allergic asthma.

Allergic reactions produce both immediate responses, which occur in minutes, as well as late responses, which occur over a period of hours or days. Late responses may result in pulmonary inflammation and mucus secretion by mechanisms that are, as yet, undetermined. Observations by Dr. Lemanske and his colleagues indicate that these reactions may contribute to the pulmonary symptoms observed in CF patients and allergic asthmatics.

In studies with rats, Dr. Lemanske and his colleagues have produced pulmonary allergic reactions and have demonstrated that these responses cause inflammation of the air passages. Further, they have isolated mast cells, which are cells in the body responsible for allergic reactions, and demonstrated that these cells contain factors that induce inflammatory changes within the lungs of these animals. These experiments indicate that allergic reactions in the lungs can lead to inflammation.

The investigators are also measuring pulmonary function in these experimental animal models to determine if the inflammatory response in any way compromises airway function. Results of these studies are providing vital insight into how allergic reactions may participate in chronic inflammatory diseases of the lungs. With this knowledge, scientists are optimistic that better treatments can be developed, not only for patients with CF, but also for patients with allergic asthma.

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Most lung infections in CF patients are caused by Pseudomonas aeruginosa. Once a Pseudomonas infection is established, it is virtually impossible to eliminate with antibiotics; therefore, an effective vaccine could prevent the tragic suffering and loss of lives caused by this virulent organism. One of the major goals of NIAID's research on CF is the development of a nontoxic Pseudomonas vaccine to be given to CF patients before their first infection by the bacterium.

An important study on the development of such a vaccine is being conducted by NIAID grantee Dr. James E. Pennington at the Brigham and Women's Hospital in Boston. He and his colleagues have demonstrated that immunization of guinea pigs with a Pseudomonas vaccine significantly protects against experimentally induced chronic Pseudomonas pneumonia. This lung protection was manifested by reduced numbers of the bacterium in the lungs of vaccinated guinea pigs and amelioration of the lung infection.

Additionally, the researchers are evaluating a variety of immunizing regimens to determine the most feasible, yet protective, method. Adverse and/or beneficial effects of giving steroids (agents that reduce inflammation) during chronic Pseudomonas lung infections are being assessed, and the interactions of LPS immunization and steroids in experimental animals are being studied.

The scientists are optimistic that the vital information furnished by these studies will provide the information necessary for planning and developing clinical trials of a Pseudomonas vaccine in CF patients.

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National Institute of General Medical Sciences

NIGMS supports research and research training in basic fields that provide the foundation for a better understanding of fundamental life processes. One of NIGMS' major programs supports basic research on genetics. This research supplies new knowledge, theories, and concepts that may lead to improved diagnostic techniques and treatments for many diseases, including CF.

While the hereditary pattern for the transmission of CF is well known, no genetic defect has yet been found that can account for the disease's symptoms. Studies to identify such a defect are facilitated by the Human Genetic Mutant Cell Repository, supported by a contract from NIGMS to the Institute for Medical Research in Camden, New Jersey. Recently, the repository has had as one of its highest priorities the establishment of a CF cell collection to aid in identifying the genetic basis of this disease. To this end, the repository has now acquired cell lines from six families, each of which contains at least three affected siblings as well as normal siblings, and the parents. This brings to 115 the number of cell lines in the repository from CF patients and unaffected family members. These cell lines, along with detailed background material, are made available to investigators at a modest charge and are among the items requested most frequently from the repository. Using these cell lines, a number of laboratories are currently attempting to correlate the expression of CF with inherited variations in certain genetic sequences, known as restriction fragment length polymorphisms (RFLP's). Finding such a correlation, or linkage, to an RFLP could permit accurate prenatal diagnosis of the defect, isolation and characterization of the gene for CF, and possible therapy.

Outlook

While research has not yet provided a cure for CF, it has provided the tools that are enabling scientists to close in on CF. Momentum has built in the last year in research in which scientists are using state-of-the-art scientific techniques to explore CF. CF's clear genetic mode of inheritance now becomes a candidate for analysis, as it is the type of disease for which genetic techniques can be the most fruitful. Through the trans-NIH CF Coordinating Committee, for which NIADDK has lead responsibility, the Institutes with an interest in CF have worked cooperatively to develop programs in support of new research initiatives. The goal of the committee and of NIH research is to continue to identify and stimulate new opportunities for studying this disease and draw on expertise developed in other fields to conquer this one. The outlook is more promising than ever.

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